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A New Fungus Disease of the White Shrimp *Penaeus setiferus*[1]

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A primary mycosis of larvae of the white shrimp, *Penaeus setiferus,* is described. The disease first became apparent in larvae in the second protozoeal stage and disappeared as the shrimp reached the first mysis stage. Affected shrimp became immobilized by near complete tissue destruction and replacement by the expanding mycelium. The fungus was found to be *Lagenidium* sp. and was infective to larval brown shrimp, *Penaeus aztecus.*

INTRODUCTION

A disease characterized by an internal fungus has recently become apparent in laboratory-reared larvae of the white shrimp, *Penaeus setiferus.* The affected larvae were hatched from the fertile eggs in the laboratory.

The presence of the disease first became apparent as the white shrimp larvae reached the second protozoeal stage. Signs of the disease disappeared and daily mortality returned to normal levels when the shrimp larvae reached the first mysis stage. Mortality in the larvae of *P. setiferus* attributed to fungal infection eached 12.4%.

Primary mycosis caused by the aquatic fungus *Saprolegnia parasitica* has been reported in larvae of the shrimp *Palaemonetes kadiakensis* reared in the laboratory (Hubschman and Schmitt, 1969). Mycoses occurring in other marine crustaceans have been recently reviewed (Johnson and Sparrow, 1961; Johnson, 1970).

The Dow Chemical Company's shrimp hatchery at Freeport, Texas, reported that a large number of brown shrimp larvae, *P. aztecus,* were infected in the spring of 1971 with a fungus, tentatively identified as belonging to the genus *Lagenidium.* The fungus caused extensive mortality within 2 or 3 days (Cook, 1971). No additional data on the fungus or the disease was given.

A preliminary description of an internal fungus, a species of *Lagenidium,* which is the cause of a primary mycosis in larvae of the white shrimp, is given here.

MATERIALS AND METHODS

White shrimp larvae infected with an internal fungus were obtained from the experimental shrimp hatchery at the Galveston Laboratory. Initial isolation of the causative fungus was made using thioglycolate media (Ray, 1966) with penicillin (500 units/ml of media) and streptomycin (500 μg/ml of media) added to inhibit bacterial growth. Whole infected shrimp larvae were introduced into the media and the cultures were incubated at room temperature (27° to 30°C). After fungal growth became evident (usually 48–72 hr) mycelial masses were removed from the media with an inoculating loop and streaked for individual colonies onto Sabouraud dextrose agar (Difco) enriched with 2% salt and 5% homogenized shrimp. Colonies of the fungus became apparent after 24–48 hr and these were serially transferred to new media until

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pure cultures of the fungus were obtained. Cultures were maintained in Sabouraud broth containing 2% salt.

Sporulation was induced in fungus cultures by transferring mycelial masses from Sabouraud broth to sterile sea water (20‰) at 28°C. Cultures of the fungus in which sporogenesis had begun were used to test the infectivity of the fungus on larval brown shrimp, *Penaeus aztecus*.

Pure cultures of the fungus were sent to Dr. A. J. Domnas, University of North Carolina, Chapel Hill, N.C. and Dr. C. E. Bland, East Carolina University, Greenville, N.C., for identification.

Results

White shrimp larvae heavily infected by an internal fungus, first became apparent at the Galveston Laboratory on July 10, 1972. The infection was limited to one tank, and mortality attributed to the disease reached 12.4%. Affected larvae were in the second protozoeal stage of larval development. Mortality stopped and signs of disease disappeared as the remaining larvae reached the first mysis stage. This was approximately 48 hr after the first signs of the disease appeared. Spores from the fungus were still present in water samples at this time.

The fungal mycelium gradually invaded and replaced nearly all the tissues within the larval shrimp. The thorax, abdomen, eye stalks, and even the swimming appendages were filled with hyphae (Figs. 1, 2). Massive tissue destruction and replacement by the fungus resulted in immobilization of the shrimp. Such larval shrimp settled to the bottom of the container when water circulation was stopped. An occasional movement of an appendage or contraction of the hindgut musculature were the only sign of life seen in these shrimp.

The fungal hyphae within the body of the larval shrimp were contorted, irregular, branched, sparingly septate, thin-walled, and averaged 8.6 to 10.7 μm in diameter (Fig. 3). The hyphae were a pale yellowish-green color and possessed numerous round refractive oil droplets (Fig. 4). A few

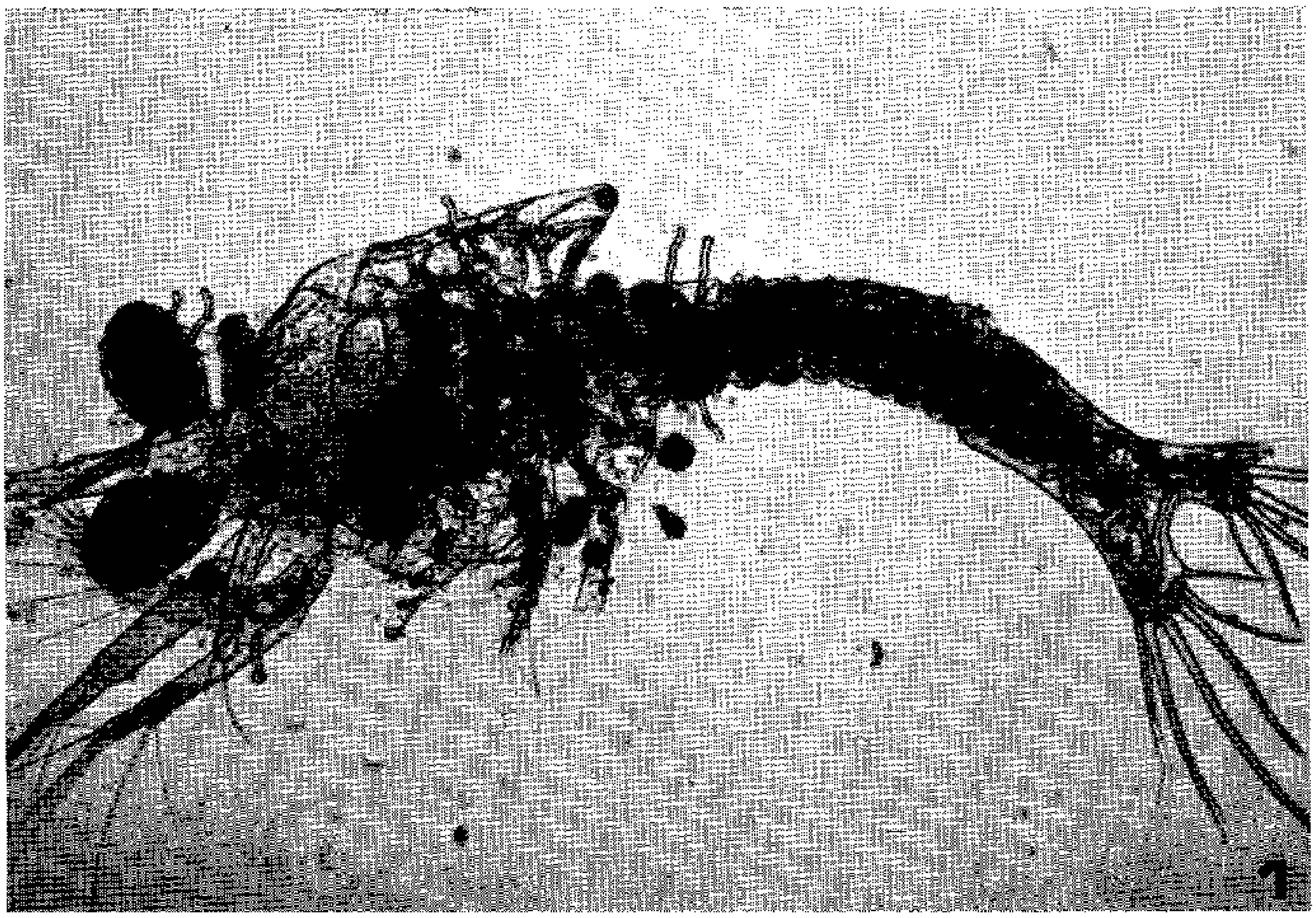

FIG. 1. Larval white shrimp (protozoeal II) heavily infected with a *Lagenidium* sp. extramatrical hyphae, some with terminal vesicles, are shown protruding from the shrimp. No stain. ×72.

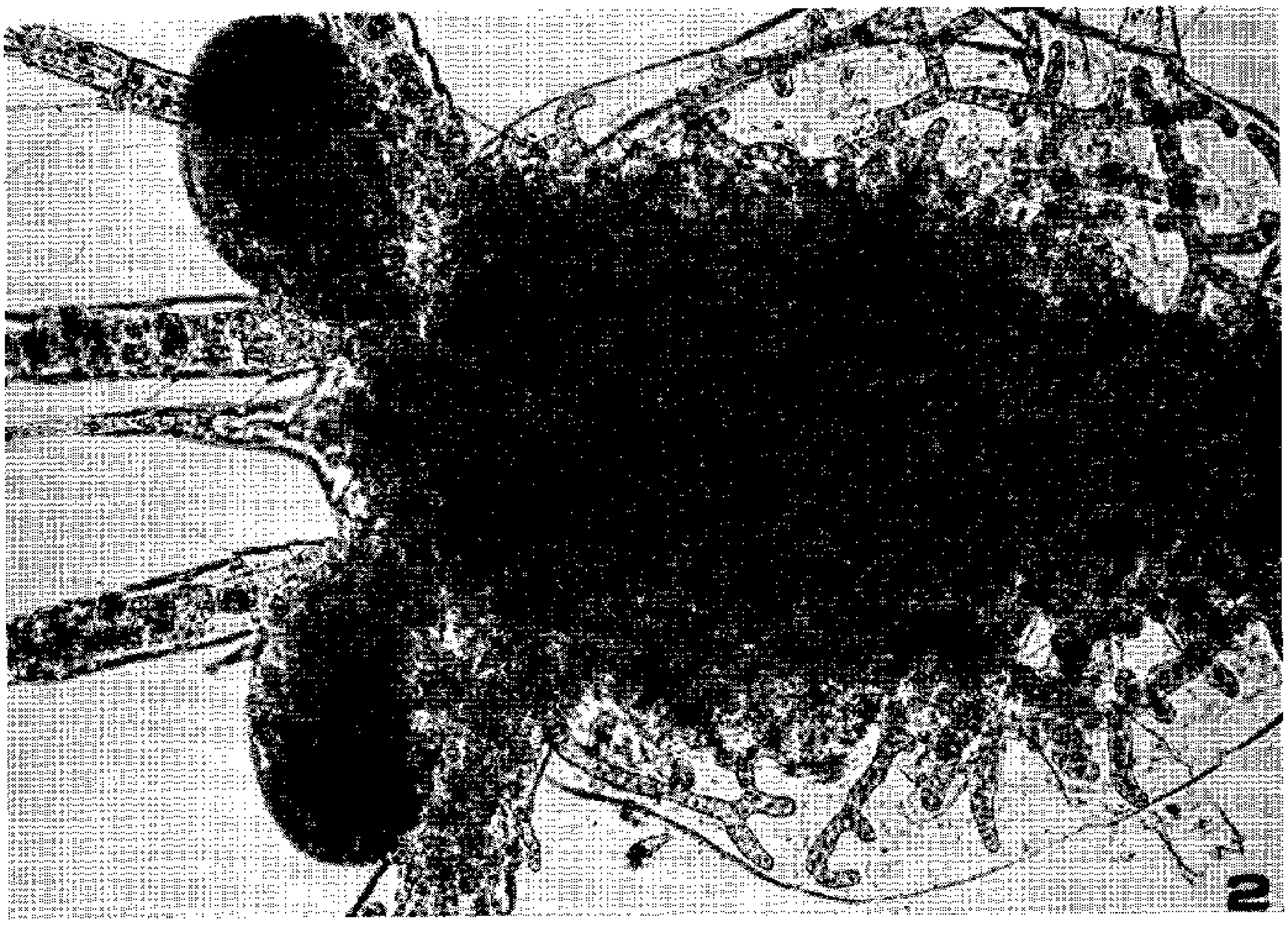

FIG. 2. Larval white shrimp with hyphae of *Lagenidium* sp. occupying much of the space in the cephalothrorax, eye stalks, and appendages. No stain. ×140.

FIG. 3. Hyphae of *Lagenidium* sp. in the cephalothorax of a larval white shrimp. The hyphae are contorted, irregular, thin-walled, and possess numerous round refractive oil droplets. No stain. ×1,000.

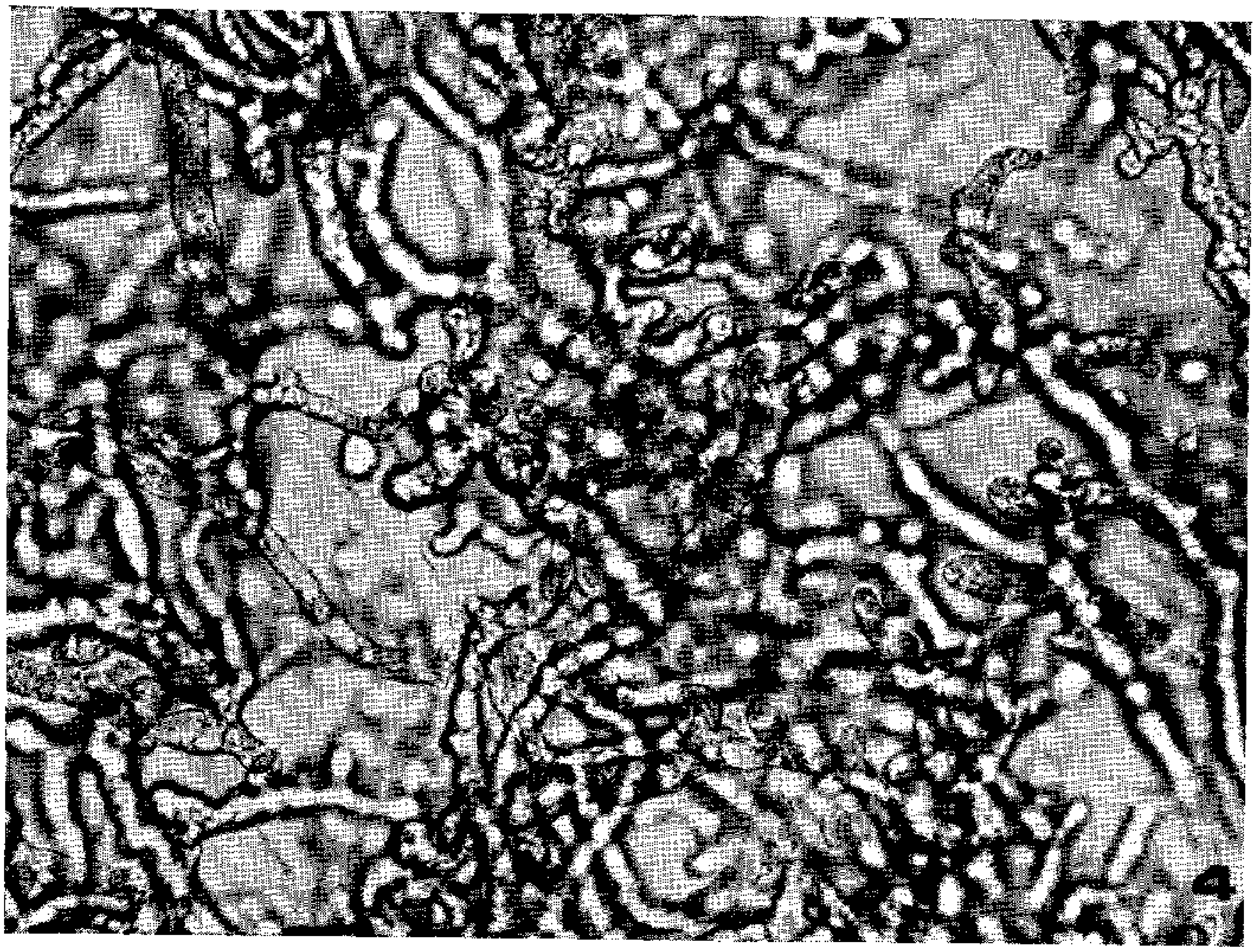

FIG. 4. *Lagenidium* sp. grown in Sabouraud broth. The hyphae are similar in appearance to hyphae in larval shrimp. No stain. ×252.

melanized areas were observed in association with the hyphae.

Sporogenesis began after the shrimp had become completely immobilized. The process of sporogenesis began with the emergence of an unbranched extramatrical "discharge tube" from the body of the shrimp (Fig. 5). The apical end of this "discharge tube" (Johnson, 1958; Bland and Amerson, 1973) swelled, forming a vesicle as it filled with individual units of cytoplasm which presumably flowed from a sporangium located on an intramaterical hypha (Figs. 6–8). At first the cytoplasm within the vesicle appeared amorphous, but gradually the outline of individual planonts became evident (Fig. 9). Soon after individual planonts became evident, the planonts began to move about slowly within the vesicle. Movement of the planonts increased until their movement caused the vesicle to rupture (Fig. 10). Reniform planonts, with two flagella originating from a lateral groove, and closely resembling those described by Couch (1942) in *L. callinectes* and Johnson (1958) in *L. Chthamaluphilum*, emerged from the vesicle opening and swam rapidly away. Individual planonts measured 8.7 by 12.0 μm. The vesicle persisted for at least a few minutes after planont discharge. The whole process of sporogenesis from appearance of the discharge tube to planont release required 30–50 min at 27°C.

The presence of fungal growth in thioglycolate medium became evident in 48–72 hr; however, growth was slow and limited in this medium. Cultures of the organism grown on Sabouraud dextrose agar enriched with 2% salt and shrimp homogenate, grew rapidly and spread over the surface of the agar, covering the plate 4–5 days after incubation at 27°C.

Cultures grew well in Sabouraud broth and the hyphae of fungus grown in this medium appeared very similar to the hyphae growing in shrimp larvae (Fig. 4).

Discharge tubes and vesicles like those in Fig. 5–9 were seen in cultures of the fungus approximately 48 hr after the fungus

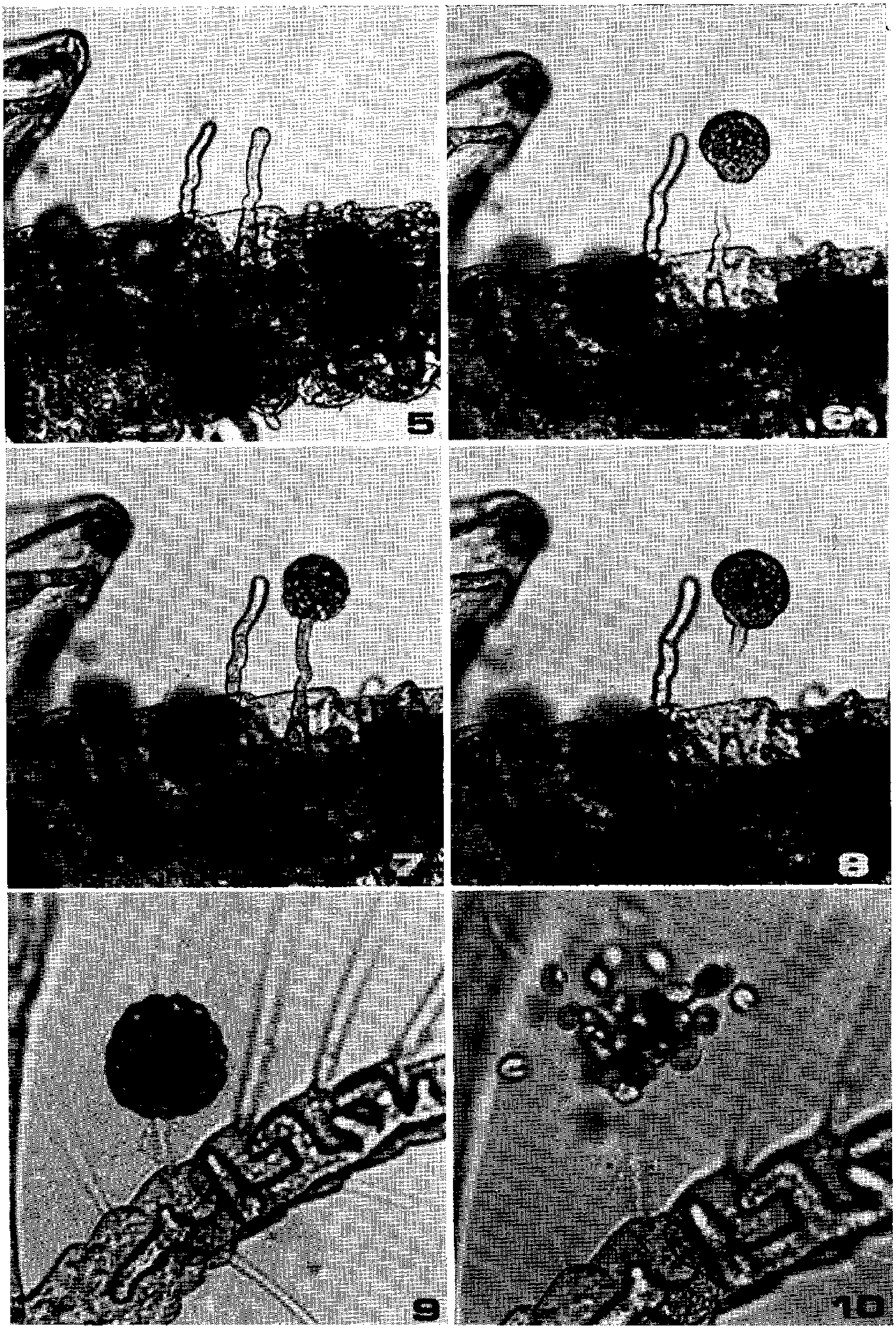

FIG. 5. An extramatrical "discharge tube" from the same shrimp as shown in Fig. 1. No stain. ×220.

FIGS. 6–8. A unit of cytoplasm is shown flowing through the "discharge tube" into the vesicle. No stain. ×252.

FIG. 9. A vesicle in which the outline of individual planonts has become apparent. No stain. ×315.

FIG. 10. The same vesicle as in Fig. 9 during planont discharge. The planonts are reniform and are motile by two flagella which arise from the lateral groove. No stain. ×800.

was transferred from Sabouraud broth to sterile sea water (salinity 28‰ at 28°C). Encystment of planonts in these preparations was observed. Planonts became spherical and cast off both flagella as they encysted.

Two thousand protozoea I larval brown shrimp, *P. aztecus*, were exposed to both planonts and hyphae of the fungus. Sixty hours later, while the shrimp were protozoea II, the fungus became apparent within some of the shrimp. By 96 hr after inoculation, approximately 5% of the shrimp examined had the disease. Mortality attributable to the fungus in experimentally infected brown shrimp larvae was approximately 20%.

Cultures of the fungus sent to Dr. A. J. Domnas and Dr. C. E. Bland were identified as belonging to the genus *Lagenidium*. However, according to Domnas and Bland this fungus differs somewhat biochemically and morphologically from *L. callinectes*. Whether or not the *Lagenidium* sp. seen in penaeid shrimp represents a new species remains to be resolved.

Discussion

Species of *Lagenidium* that are similar to the one described here occur in other marine crustaceans. *L. callinectes* occurs in the eggs (Couch, 1942) and larvae (Rogers-Talbert, 1948) of the blue crab *Callinectes sapidus*, and *L. chthamalophilum* occurs in the eggs of the barnacle *Chthamalus fragilis* (Johnson, 1958).

A species of *Lagenidium* has been reported to be the cause of high mortality in larvae of the brown shrimp, *P. aztecus*, (Cook, 1971). That fungus and the one described here may be very similar in that the isolate from white shrimp, *P. setiferus*, was infective to brown shrimp larvae.

The only other primary mycosis reported in shrimp was caused by *Saprolegnia parasitica* in larvae of the shrimp *Palaemonetes kadiakensis* (Hubschman and Schmitt, 1969). Pure cultures of that fungus were found to attack and kill healthy shrimp larvae. All larvae exposed to *Saprolegnia* developed severe localized infections, as contrasted to the total body involvement seen in white and brown shrimp larvae infected with *Langenidium* sp.

Acknowledgments

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References

Bland, C. E., and Amerson, H. V. 1973. Observations on *Lagenidium callinectes* Couch: Isolation and sporangial development. *Mycologia*, in press.

Cook, H. L. 1971. Fungi parasitic on shrimp. *FAO Aquacult. Bull.* **3**, 13.

Couch, J. H. 1942. A new fungus on crab eggs. *J. Elisha Mitchell Sci. Soc.*, **58**, 158–162.

Hubschman, J. H., and Schmitt, J. A. 1969. Primary mycosis in shrimp larvae. *J. Invertebr. Pathol.*, **13**, 351–357.

Johnson, T. W. 1958. A fungus parasite in ova of the barnacle *Chthamalus fragilis denticulata*. *Biol. Bull.*, **114**, 205–214.

Johnson, T. W., Jr. 1970. Fungi in marine crustaceans. *In* "A Symposium on Diseases of Fishes and Shellfishes" (S. F. Snieszko, ed.), *Amer. Fish. Soc. Spec. Publ.* No. 5, pp. 405–408.

Johnson, T. W., Jr., and Sparrow, F. K., Jr. 1961. "Fungi in Oceans and Estuaries." Cramer, Weinheim. 668 pp.

Ray, S. M. 1966. Effects of various antibiotics on the fungus *Dermocystidium marinum* in thioglycollate cultures of oyster tissues. *J. Invertebr. Pathol.*, **8**, 433–438.

Rogers-Talbert, R. 1948. The fungus *Lagenidium callinectes* Couch (1942) on eggs of the blue crab in Chesapeake Bay. *Biol. Bull.*, **95**, 214–228.